Job No.: 1505-80958
Translated from Japanese by the Ralph McElroy Translation Company
910 West Avenue, Austin, Texas 78701 USA

INTERNATIONAL PATENT OFFICE
WORLD ORGANIZATION FOR INTELLECTUAL PROPERTY
International patent published on
the basis of the Patent Cooperation Treaty
INTERNATIONAL PUBLICATION NO. WO 00/09557 A1

International Patent Classification[6]:	C 07 K 14/47 C 12 N 15/12 //5/10 C 12 P 21/02 (C 12 P 21/02 C 12 R 1:91)
International Filing No.:	PCT/JP99/04352
International Filing Date:	August 11, 1999
International Publication Date:	February 24, 2000
Priority Date: Country: No.:	 August 12, 1998 JP Hei 10[1998]-227723
Designated States:	AU, CA, CN, KR, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE)

NOVEL GENE AND PGTH PROTEIN ENCODED THEREBY

Inventors and
Inventors/Applicants (only for US):

Osamu Ohara;
Takahiro Nagase;
Nobuo Nomura
Kazusa DNA Research Institute
Foundation
1532-3 Yakuni, Kisarazu-shi,
Chiba-ken (JP)

Kiyoshi Takayama
Hitoshi Toyoda;
Makoto Yoshimoto
Taisho Pharmaceutical Co., Ltd.
3-24-1 Takata, Toshima-ku,
Tokyo-to (JP)

Applicant (for all designated states other than US):

Kazusa DNA Research Institute Foundation
1532-3 Yakuni
Kisarazu-shi, Chiba-ken (JP)

Taisho Pharmaceutical Co., Ltd.
3-24-1 Takata, Toshima-ku,
Tokyo-to (JP)

Agent:

Tomizo Kitagawa
Taisho Seiyaku K.K.
3-24-1 Takata, Toshima-ku,
Tokyo-to (JP)

Published
With International Search Report.

FOR INFORMATION ONLY

Codes for the identification of PCT contract states on the cover sheets of the documents that publish the international applications in accordance with the PCT.

AE	United Arab Emirates
AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia-Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CR	Costa Rica
CU	Cuba
CY	Cyprus
CZ	Czech Republic
DE	Germany
DK	Denmark
DM	Dominica
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GD	Grenada
GE	Georgia
GH	Ghana
GM	Gambia
GN	Guinea
GR	Greece
HR	Croatia
HU	Hungary
ID	Indonesia
IE	Ireland
IL	Israel
IN	India
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	South Korea
KZ	Kazakhstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MA	Morocco
MC	Monaco
MD	Republic of Moldavia
MG	Madagascar
MK	Macedonia (former Yugoslavian Republic of Macedonia)
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SL	Sierra Leone
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
TZ	Tanzania
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Vietnam
YU	Yugoslavia
ZA	South Africa
ZW	Zimbabwe

Field of the technology

This invention pertains to a novel PGTH protein of human brain origin having a prostaglandin transport activity and the pgth gene encoding the protein.

Prior art

Prostaglandin is a generic name for a series of physiologically active lipids such as prostaglandin E, prostaglandin D, prostaglandin F, prostaglandin I, prostaglandin J, etc. Prostaglandin is a physiologically active substance inside the body strongly related to control of physiological functions such as blood flow rate, sleeping, gastric mucosa protective action, thrombus formation, pregnancy, etc., through specific cell membrane or intranuclear receptors.

Prostaglandin is produced inside cells as a result of eicosapolyenic acids such as arachidonic acid, etc., being cut out by phospholipase A2 from the cell membrane and converted with cyclooxygenase and various prostaglandin synthetic enzymes by responding to various physiological stimuli, and after being released outside the cells, it has autocrine or paracrine effects. On the other hand, liberated prostaglandin is also circulated in the blood flow, taken up by a specific cell, metabolized and so disappears.

A trace amount of prostaglandin shows a strong physiological activity, and consequently, the production of prostaglandin compounds is strictly controlled by controlling the activity of production-related and metabolism-related enzymes.

However, prostaglandin has been reported to be unable to pass through the lipid double layer of the cell membrane by itself. Therefore, as a prostaglandin transport mechanism, the presence of a special protein has been presumed in the process of prostaglandin produced inside a cell exiting it and the process of prostaglandin circulating in the blood flow being taken up into a specific cell.

As a protein involved in the transport mechanism described above, prostaglandin transporter (abbreviated hPGT: human prostaglandin transporter, below) has been reported, but it is not a protein involved in the transport of all prostaglandin compounds, and there are many unclear points. Consequently, it is thought that if a biological molecule other than hPGT involved in the transport mechanism can be elucidated, the biological molecule found might be usable directly as a medical drug or indirectly as a compound for studying compounds that might be usable as a medical drug. Therefore, the objective of this invention is to identify such a molecule and use it as a medical drug or for the development of medical drugs.

Presentation of the invention

The inventors of this invention studied diligently to find the desired protein by using genes expressed in the human brain, and as a result, they found the presence of a novel PGTH protein (prostaglandin transporter homologue), successfully isolated a pgth gene encoding the protein, and they arrived at this invention.

Specifically, this invention pertains to (a) a protein having the amino acid sequence described in sequence No. 1 or (b) a protein having an amino acid sequence with 1 to several amino acids deleted, substituted or added to the amino acid sequence of sequence No. 1, and having a prostaglandin transport activity.

Furthermore, this invention also pertains to (c) a gene comprising DNA described sequence No. 2 or (d) DNA which can be hybridized with the DNA of sequence No. 2 under stringent conditions and which encodes a protein having a prostaglandin transport activity.

The pgth gene of this invention can be isolated as a cDNA fragment containing the gene from a cDNA library of human brain origin. The cDNA library used by the inventors of this invention was prepared based on commercially available mRNA of human brain origin from the Clonetech Co.

As a method for identifying the cDNA encoding a protein having a prostaglandin transport activity in the cDNA library described above, the method of Ohara, et al., (DNA Research 4: p 53, 1997) was used as an extensive cDNA library analysis method using a long-chain cDNA library. From a long-chain cDNA library of human brain origin prepared by the method of Ohara, et al., 25,000 recombinants are randomly selected, the 5' and 3' - base sequences of the cDNA from 15,000 clones were determined, and a clone showing homology to the gene encoding hPGT already reported from the 5' sequences of all the clones can be found by using a DNA analysis program (BLAST and FastA).

The presence of a region encoding the protein (ORF: open reading frame) in the base sequence can be confirmed by a conventional method using a computer program. After becoming confident of the presence of the desired gene in the cDNA sequence, the inventors of this invention found one ORF in the sequence by utilizing a computer, the gene was named pgth, and the protein encoded by the gene was named PGTH. The PGTH of the invention is a protein comprising a total of 709 amino acid residues and having a molecular weight of about 80 kd.

The invention pgth is a gene comprising 2130 bp shown in sequence No. 2. By using this pgth and conventional genetic recombination techniques using a suitable host vector system, it is possible to prepare a recombinant gene. As a suitable vector, there are plasmids of *E. coli* origin (such as pBR322, pUC118, etc.), of *Bacillus subtilis* origin, (such as pSH19, etc.) yeast origin plasmid (such as pUB110, pC194, etc.), bacteriophages, animal viruses such as retroviruses,

vaccinia virus, etc., etc. At the time of recombination, it is possible to add translation initiation and termination codons using suitable DNA adaptors. Furthermore, for gene expression, a suitable expression promoter is attached upstream of the gene. The promoter to be used is suitably selected depending on the host used. For example, if the host is *E. coli*, there are T7, lac, trp, λPL promoters, etc.; if the host is a *Bacillus*, there are SPO promoters, etc.; if the host is a yeast, there are PHO5, GAP, ADH promoters, etc.; and if the host is an animal cell, there are SV40-origin, retrovirus promoters, etc.

Furthermore, the gene may be expressed as a fused protein with another protein (such as glutathione-S-transferase, protein A, etc.). In the case of a fused PGTH prepared by using such a method, a suitable protease (such as thrombin, etc.), may be used to cut out the protein.

As a host usable in the case of PGTH expression, there are various strains of *Escherichia coli*, various strains of *Bacillus subtilis*, various strains of the yeast *Saccharomyces cerevisiae* and animal cells such as COS-7, CHO cells, etc.

As a method for transforming a host cell using the above recombinant vector, a specific method conventionally used to transform the selected host cell is used.

Incidentally, in this invention, DNA which has a DNA sequence other than that shown in sequence No. 2 which can be hybridized with the DNA and encodes a protein having a prostaglandin transport activity, is also included in the scope of this invention.

Specifically, DNA which has a DNA sequence, the total length of the pgth sequence, partially changed due to various artificial treatments such as random mutations, introduction of site-specific mutations, or mutagen treatment, DNA fragment mutation, deletion ligation after scission with restriction enzymes, is also included in the scope of this invention in spite of having a DNA sequence different from that of sequence No. 2 as long as such a DNA variant can be hybridized with pgth under stringent conditions and encodes a protein having a prostaglandin transport activity.

The extent of the above DNA mutation is within the allowable range if the variant has 90% or higher homology with the DNA sequence of pgth. Furthermore, as an extent of hybridization with pgth, Southern hybridization with pgth may be carried out under conventional conditions, for example, in the case of probe labeling with a DIG DNA Labeling kit (Boehringer-Mannheim Cat. No. 1175033), hybridization conditions of a DIG Easy Hyb solution (Boehringer-Mannheim Cat. No. 1603558) at 32°C and washing of the membrane in a 5X SSC solution (containing 0.1% w/v SDS) at 50°C (1X SSC comprises 0.15M NaCl and 0.015M sodium citrate).

Furthermore, a protein encoded by the gene variant which is highly homologous to pgth as described above and has a prostaglandin transport activity is also included in the scope of this invention.

Specifically, a variant having one or more amino acids deleted, substituted or added to the amino acid sequence of PGTH is included in the scope of this invention as long as this variant is a protein having a prostaglandin transport activity.

The side chains of the amino acids, which are the constituent elements of proteins are respectively different with respect to hydrophobicity, electrical charge, size, etc., but several highly conservative relationships in the meaning of practically not affecting the three-dimensional structure (it is also called the steric structure) of proteins have been known from experiences or actual physicochemical observations. For example, for substitution of amino acid residues, there are glycine (Gly) and proline (Pro), Gly and alanine (Ala) or valine (Val), leucine (Leu) and isoleucine (Ile), glutamic acid (Glu) and glutamine (Gln), aspartic acid (Asp) and asparagine (Asn), cysteine (Cys) and threonine (Thr), Thr and serine (Ser) or Ala, lysine (Lys) and arginine (Arg), etc.

Therefore, any variant protein due to substitution, insertion, deletion, etc., in the amino acid sequence of the PGTH shown in sequence No. 1 can be said to be within the scope of this invention if the variation is a variation which conserves the three-dimensional structure of the PGTH, and the protein is a protein having a prostaglandin transport activity similar to PGTH. The allowable extent of this variation is 90% or higher homology with the amino acid sequence shown in sequence No. 1.

Industrial application field

The abnormal expression of pgth or functional failure of PGTH is presumed to be a critical disorder because PGTH has a prostaglandin transport activity, and consequently the normal prostaglandin production mechanism of the body is lost.

Therefore, PGTH itself is considered to be useful as a drug, and on the other hand, pgth or PGTH may be used for effectively studying or evaluating a substance having the same function as that of PGTH, a substance promoting or inhibiting its function, a substance promoting the expression of the gene, etc.

Best embodiment of the present invention

This invention is explained further in detail using application examples as follows, but this invention is certainly not limited to these application examples. Incidentally, unless specified, the experimental procedures used in the following application examples are those

described in standard experimental manuals such as Molecular Cloning, 2nd ed. (Cold Spring Harbor Laboratory Press, 1989), etc., and the operating manuals in commercially available kits, and they can be carried out under the conditions recommended for the respective commercially available products such as restriction enzymes, etc.

Application Example 1 Cloning of pgth

1) Construction of a long chain cDNA library of human brain origin

An oligonucleotide (GACTAGTTCTAGATCGCGAGCGGCCGCCC$(T)_{15}$) containing a NotI site was synthesized using a DNA synthesizer (ABI380B). It was used as a primer, and a double chain cDNA was synthesized using mRNA of human brain origin as a template and the SuperScript II reverse transcriptase kit (Gibco BRL). The ligation of the synthetic DNA was carried out with the cDNA and SalI site-containing adapter (Takara Shuzo), subsequently, NotI digestion was carried out, and cDNA fragments of 3 kb or larger were purified using electrophoresis with a 1% concentration of low-melting agarose.

After ligation of the purified cDNA fragments with a SalI-NotI restriction enzyme-treated pBluescriptIISK+ plasmid, the recombinant plasmids were introduced into *E. coli* ElectroMax DH10B strain (Gibco BRL) using the electroporation method. Subsequently, 25,000 recombinants were randomly selected from the library, the recombinant DNAs were extracted, and the 5'- and 3'-base sequences of the cDNAs of 15,000 clones were determined. For the sequence determination, a PE Applied Biosystem Co., DNA sequencer (ABI PRISM377) and the reaction kit from the same company were used.

2) Selection of clones containing the pgth sequence

The 5 sequences of all the clones determined in 1) were compared with the sequence of hPGT already reported using DNA analytical programs (BLAST and FastA), and as a result, a clone named HK07457 showed significant homology.

3) DNA fragment base sequence determination

The base sequence determination was carried out using a PE Applied Biosystem Co. DNA sequencer and the dye primer method. The sequence was mostly determined using the shotgun method, and for a portion of the base sequence, an oligonucleotide was synthesized based on the base sequence already determined, and the primer walking method was used to determine the entire base sequences of the two chains. The entire base sequence of the cDNA of the clone is shown in sequence No. 3.

The cDNA contains an ORF encoding a protein (PGTH) comprising 709 residues. A termination codon was found to appear in the upstream region of a methionine residue, which was an initiation codon of the protein, with the same reading frame. Therefore, the amino acid sequence shown in sequence No. 3 was confirmed to be the only possibility as an amino acid sequence of the protein encoded by the cDNA fragment.

Figure 1 shows the amino acid homology between already reported hPGT and the PGTH of this invention. The two show high homology, especially, the position of the cysteine residue present at the C-terminal of PGTH is preserved, and the 77th residue glutamine, 561st residue arginine and 614th residue lysine of hPGT, which are amino acids especially important for the transport activity, are also preserved in PGTH.

Application Example 2

Confirmation of protein expression by in vitro translation of pgth

The plasmid containing pgth prepared in Application Example 1 was treated with RNase A, subsequently, RNase A was removed using ADVAMAX beads (AGTC Co.), and in vitro translation was carried out using a TNT T7 coupled reticulocyte lysate system (Promega Co.) in the presence of (^{35}S)-methionine. A portion of the reaction mixture was separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and the analysis carried out using BAS-2000 (Fuji Shashin Kogyo). As a result, the presence of a single band at about 80 kd was confirmed as shown in Figure 2.

Application Example 3

Construction of animal cell expression vector

1) Amplification of ORF-containing cDNA

An oligonucleotide (following sequence 1) having a sequence upstream from the initiation codon of the protein of sequence No. 3 and oligonucleotide (following sequence 2) having a sequence of a portion downstream from the termination codon of the protein and the reverse complementary strand chain were synthesized using a DNA synthesizer (ABI Co., Model 380B).

Sequence 1

5-CTGGAGCTCACTGCACTCCAGCAGTC-3

Sequence 2

5-AGCTCACACTCGGGAATCCTCTGGCTTC-3

The recombinant cDNA containing sequence No. 3 isolated in application example 1 was used as a template, the oligonucleotides of the sequences 1 and sequences 2 were used as a primer, and the following PCR procedures were carried out using a Takara LA PCR kit Ver. 2 and the PCR thermal cycler MP (Takara Shuzo).

cDNA	5 μL (10 ng)
10X PCR buffer (containing 25 mM Mg^{++})	5 μL
2.5 mM dNTP	8 μL
10 μM Sequence 1	2 μL
10 μM Sequence 2	2 μL
Water	27.5 μL
LA Taq polymerase	0.5 μL
Total amount	50 μL

The PCR cycle was carried out by holding at 94°C for 2 mn, carrying out the reaction at 98°C for 20 sec, cooling to 68°C at a rate of 1°C/2 sec, holding at 68°C for 3 min, at 72°C for 10 min, and repeating 30 times.

The above method was used to amplify a DNA fragment (about 2.2 kb) having a portion of sequence No. 3.

2) Subcloning to an animal cell expression vector

The DNA fragment amplified in 1) was fractionated by 1% agarose gel electrophoresis. After staining the gel with ethidium bromide, the gel containing the desired band observed under ultraviolet irradiation was cut out. The extraction of the DNA fragment from the agarose gel and purification were carried out using a GENECLEAN II Kit (Bio101 Co.)

The extracted and purified DNA fragment was subcloned to animal cell expression vector pTARGET (Promega Co.) The ligation solution used was a Takara Ligation Kit Ver. 2 (Takara Shuzo), and the reaction was carried out with the following composition at 16°C for 1.5 h.

Extracted and purified DNA fragment	1 μL (50 ng)
PTARGET	1 μL (10 ng)
Water	3 μL
Ligation solution	5 μL
Total	10 μL

The reaction solution after the above reaction was used to transform the *E. coli* K12 strain DH5. The transformant was inoculated on an LB agar medium containing 50 μg/mL of ampicillin (Amp), 40 μg/mL of 5-bromo-4-chloro-3-indolyl-β-D-galactoside (IPTG) [sic; isopropyl-β-D-thioglucopyranoside] and 100 μM of isopropyl-b-D-thiogalactopyranoside

(X-gal) [sic; 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside] *and cultivated overnight at 37°C.

Each colony that developed on the above plate was inoculated in 10 mL of an LB liquid medium containing 50 μg/mL of Amp, cultivation was carried out overnight at 37°C, the biomass was collected by centrifugation, and subsequently the recombinant DNA was purified using a QIAprep Spin Plasmid Miniprep Kit (Qiagen Co.) to obtain pTARGETpgth.

3) Determination of the base sequence of the inserted cDNA

The base sequence determination was carried out using a DNA sequencer (ABI Co., Model PRISM377) and the dye terminator method, and the whole base sequence of the two chains was determined using the primer walking method. The clone was found to contain all of the region between sequences 1 and 2 among sequence No. 3 confirming that the desired gene pTARGETpgth had been cloned.

Application Example 4

Insertion into CHOk1 cells and stable transformant preparation

The recombinant DNA, pTARGETpgth, prepared in Application Example 2 has a CMV promoter upstream of pgth, and if it is inserted into an animal cell, the expression of pgth is possible.

CHOk1 cells were cultured in 60 mm diameter plastic Petri dishes. As the culture medium, Ham F-12 (Gibco, called growth medium, below) containing 10% fetal bovine serum (Dainippon Seiyaku), 50 U/mL of penicillin and 50 μg/mL of streptomycin was used, and culture was carried out at 37°C in the presence of 5% CO_2. When the cell density was 50%, LIPOFECTAMINE reagent (Gibco) containing pTARGETpgth prepared in Application Example 2 was added in a layer over the cells, incubated for 6 h, and, after replacement with the growth medium, culture was continued for 48 h. After dispersing the cells with trypsin, the cell suspension was placed in a 60 mm diameter plastic Petri dish, and culture was carried out for 24 h. After removing the culture medium, it was replaced by growth medium containing G418 (Gibco, final concentration of 500 μg/mL). The G418 medium was changed every 3 days and culture continued for 2 weeks. When the cell colonies were observable with the naked eye, 3 colonies were isolated using stainless steel cups. As a control, only the pTARGET vector (Promega Co.) was inserted into CHOk1 cells by carrying out the same procedures as those described above to isolate a stable transformant.

---

* [Editor's note: The compound names and abbreviations are so garbled in the original text that it is impossible to be certain whether it should be 40 μg/mL IPTG and 100 μm X-gal, or vice-versa.]

washed with a suitable buffer solution containing bovine serum albumin, and culture was continued for 20 min using a buffer solution containing (^{3}H)-labeled PGE2 (Amersham Co.). After washing the cells, they were recovered, and the radioactivity taken up was measured. As a result, the prostaglandin transport activity of the CHOk1 cells with pgth inserted was statistically significantly higher than that of the CHOk1 cells with only the control vector inserted.

Application Example 6

Expression of pgth mRNA in human macrophages loaded with oxidized LDL

1) Preparation of human macrophages loaded with oxidized LDL and normal monocyte cDNA

Normal monocyte cDNA was prepared using RNA prepared with Trizol (Gibco BRL Co.) from CD14-positive monocytes from human peripheral blood as a template and the SuperScript II reverse transcriptase kit (Gibco BRL). Human macrophages loaded with oxidized LDL were prepared by culturing normal monocytes in a RPMI-1640 medium (Dainippon Seiyaku) containing 20% AB serum and antibiotics for 14 days, adding human LDL oxidized with copper sulfate using conventional procedures (oxidized LDL) in the final concentration of 40 μ/mL [sic; dimension incorrect] and continuing culture for 24 h. A method similar to that used for normal monocytes was used to prepare cDNA.

2) Confirmation of pgth mRNA expression by the RT-PCR method

Oligonucleotides (following sequence 3) having a sequence contained in sequence No. 2 and oligonucleotides (following sequence 4) having the sequence of the reverse complementary strand were respectively synthesized using a DNA synthesizer (ABI Co., Model 380B).

Sequence 3

5-GCTCCTGCCCATTGGACGGCTTTAACC-3

Sequence 4

5-TCACACTCGGGAATCCTCTGGCTTC-3

The cDNA prepared in (1) was used as a template, the oligonucleotides with sequences 3 and 4 were used as primers, and the following PCR procedures were carried out using a Takara LA PCR kit Ver. 2 and the PCR thermal cycler MP (Takara Shuzo).

cDNA	2 μL (40 ng)
10X PCR buffer (containing 25 mM Mg^{++})	1.5 μL
2.5 mM dNTP	2.4 μL

10 μM Sequence 3	0.4 μL
10 μM Sequence 4	0.4 μL
Water	10.15 μL
LA Taq polymerase	0.15 μL
Total amount	15 μL

The PCR cycle was carried out by holding at 94°C for 5 min, carrying out the reaction at 94°C for 1 min, holding at 58°C for 1 min, furthermore at 72°C for 1 min, and repeating 30 times. The PCR reaction mixture was fractionated using 1% agarose gel electrophoresis. After staining the gel with ethidium bromide, the ultraviolet irradiation was carried out to detect an amplified band at about 500 bp. Similarly, the glyceraldehyde 3-phosphate dehydrogenase gene amplified primer (G3PDH, Clonetech Co.) was used as the standard cDNA for PCR testing. As a result, the expression of pgth mRNA was strongly induced in the macrophages loaded with oxidized LDL, as shown in Figure 3.

Normal monocytes, macrophages loaded with oxidized LDL or equivalent cultured cells may be cultured with a test compound added, and subsequently the change in the PGTH mRNA may be measured by the method described above to screen any substance controlling PGTH mRNA expression.

Brief description of the figures

Figure 1 shows comparison of amino acid sequence homology between hPGT and the PGTH of this invention.

Figure 2 shows the results of SDS-PAGE of PGTH expressed using the in vitro translation method using pgth.

Figure 3 shows the results of detection of mRNA for the expression of pgth in human macrophages loaded with oxidized LDL using the RT-PCR method. In the figure o shows the results for human macrophages loaded with oxidized LDL, and m shows the results for normal human monocytes.

Claims

(1) A protein of the following (a) or (b).

(a) Protein comprising the amino acid sequence of sequence No. 1

(b) Protein comprising an amino acid sequence with one or more amino acids deleted, substituted or added to the amino acid sequence of sequence No. 1, and, at the same time, having a prostaglandin transport activity.

(2) DNA of the following (a) or (b).

(a) DNA comprising the base sequence of sequence No. 2

(b) DNA which can be hybridized with the DNA of sequence No. 2 under stringent conditions and at the same time, encodes a protein having a prostaglandin transport activity.

Figure 1

Replacement Sheet (Regulation 26)

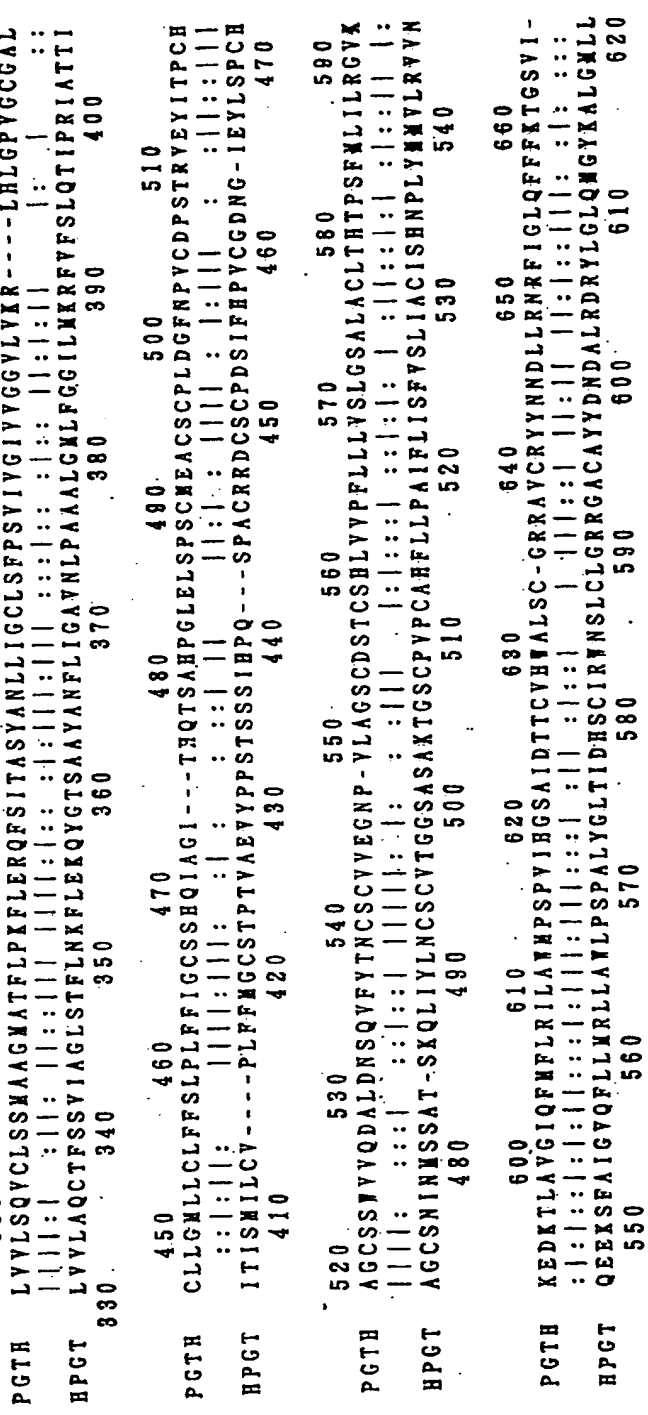

Figure 1 (cont.)

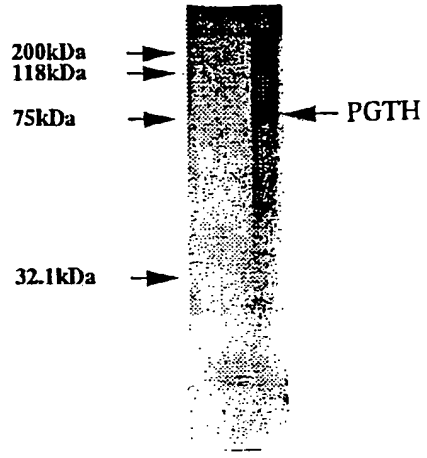

Figure 2

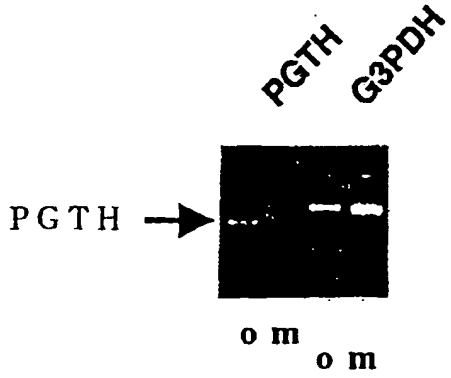

Figure 3

SEQUENCE LISTING

<110> TAISHO PHARMACEUTICAL CO., Ltd.

<120> Prostaglandin

<130> P487

<150> JP10-227723

<151> 1998-08-12

<160> 3

<210> 1
<211> 709
<212> PRT
<213> Homo sapience

<400> 1

```
Met Gly Pro Arg Ile Gly Pro Ala Gly Glu Val Pro Gln Val Pro
                  5                  10                  15
Asp Lys Glu Thr Lys Ala Thr Met Gly Thr Glu Asn Thr Pro Gly
                 20                  25                  30
Gly Lys Ala Ser Pro Asp Pro Gln Asp Val Arg Pro Ser Val Phe
                 35                  40                  45
His Asn Ile Lys Leu Phe Val Leu Cys His Ser Leu Leu Gln Leu
                 50                  55                  60
Ala Gln Leu Met Ile Ser Gly Tyr Leu Lys Ser Ser Ile Ser Thr
                 65                  70                  75
Val Glu Lys Arg Phe Gly Leu Ser Ser Gln Thr Ser Gly Leu Leu
                 80                  85                  90
Ala Ser Phe Asn Glu Val Gly Asn Thr Ala Leu Ile Val Phe Val
                 95                 100                 105
Ser Tyr Phe Gly Ser Arg Val His Arg Pro Arg Met Ile Gly Tyr
                110                 115                 120
Gly Ala Ile Leu Val Ala Leu Ala Gly Leu Leu Met Thr Leu Pro
                125                 130                 135
His Phe Ile Ser Glu Pro Tyr Arg Tyr Asp Asn Thr Ser Pro Glu
                140                 145                 150
Asp Met Pro Gln Asp Phe Lys Ala Ser Leu Cys Leu Pro Thr Thr
                155                 160                 165
Ser Ala Pro Ala Ser Ala Pro Ser Asn Gly Asn Cys Ser Ser Tyr
                170                 175                 180
Thr Glu Thr Gln His Leu Ser Val Val Gly Ile Met Phe Val Ala
                185                 190                 195
```

Gln Thr Leu Leu Gly Val Gly Gly Val Pro Ile Gln Pro Phe Gly
200 205 210
Ile Ser Tyr Ile Asp Asp Phe Ala His Asn Ser Asn Ser Pro Leu
215 220 225
Tyr Leu Gly Ile Leu Phe Ala Val Thr Met Met Gly Pro Gly Leu
230 235 240
Ala Phe Gly Leu Gly Ser Leu Met Leu Arg Leu Tyr Val Asp Ile
245 250 255
Asn Gln Met Pro Glu Gly Gly Ile Ser Leu Thr Ile Lys Asp Pro
260 265 270
Arg Trp Val Gly Ala Trp Trp Leu Gly Phe Leu Ile Ala Ala Gly
275 280 285
Ala Val Ala Leu Ala Ala Ile Pro Tyr Phe Phe Phe Pro Lys Glu
290 295 300
Met Pro Lys Glu Lys Arg Glu Leu Gln Phe Arg Arg Lys Val Leu
305 310 315
Ala Val Thr Asp Ser Pro Ala Arg Lys Gly Lys Asp Ser Pro Ser
320 325 330
Lys Gln Ser Pro Gly Glu Ser Thr Lys Lys Gln Asp Gly Leu Val
335 340 345
Gln Ile Ala Pro Asn Leu Thr Val Ile Gln Phe Ile Lys Val Phe
350 355 360
Pro Arg Val Leu Leu Gln Thr Leu Arg His Pro Ile Phe Leu Leu
365 370 375
Val Val Leu Ser Gln Val Cys Leu Ser Ser Met Ala Ala Gly Met
380 385 390
Ala Thr Phe Leu Pro Lys Phe Leu Glu Arg Gln Phe Ser Ile Thr
395 400 405
Ala Ser Tyr Ala Asn Leu Leu Ile Gly Cys Leu Ser Phe Pro Ser
410 415 420
Val Ile Val Gly Ile Val Val Gly Gly Val Leu Val Lys Arg Leu
425 430 435
His Leu Gly Pro Val Gly Cys Gly Ala Leu Cys Leu Leu Gly Met
440 445 450
Leu Leu Cys Leu Phe Phe Ser Leu Pro Leu Phe Phe Ile Gly Cys
455 460 465
Ser Ser His Gln Ile Ala Gly Ile Thr His Gln Thr Ser Ala His
470 475 480
Pro Gly Leu Glu Leu Ser Pro Ser Cys Met Glu Ala Cys Ser Cys
485 490 495
Pro Leu Asp Gly Phe Asn Pro Val Cys Asp Pro Ser Thr Arg Val
500 505 510
Glu Tyr Ile Thr Pro Cys His Ala Gly Cys Ser Ser Trp Val Val
515 520 525
Gln Asp Ala Leu Asp Asn Ser Gln Val Phe Tyr Thr Asn Cys Ser
530 535 540
Cys Val Val Glu Gly Asn Pro Val Leu Ala Gly Ser Cys Asp Ser
545 550 555
Thr Cys Ser His Leu Val Val Pro Phe Leu Leu Leu Val Ser Leu
560 565 570

```
Gly Ser Ala Leu Ala Cys Leu Thr His Thr Pro Ser Phe Met Leu
                575                 580                 585
Ile Leu Arg Gly Val Lys Lys Glu Asp Lys Thr Leu Ala Val Gly
                590                 595                 600
Ile Gln Phe Met Phe Leu Arg Ile Leu Ala Trp Met Pro Ser Pro
                605                 610                 615
Val Ile His Gly Ser Ala Ile Asp Thr Thr Cys Val His Trp Ala
                620                 625                 630
Leu Ser Cys Gly Arg Arg Ala Val Cys Arg Tyr Tyr Asn Asn Asp
                635                 640                 645
Leu Leu Arg Asn Arg Phe Ile Gly Leu Gln Phe Phe Phe Lys Thr
                650                 655                 660
Gly Ser Val Ile Cys Phe Ala Leu Val Leu Ala Val Leu Arg Gln
                665                 670                 675
Gln Asp Lys Glu Ala Arg Thr Lys Glu Ser Arg Ser Ser Pro Ala
                680                 685                 690
Val Glu Gln Gln Leu Leu Val Ser Gly Pro Gly Lys Lys Pro Glu
                695                 700                 705
Asp Ser Arg Val
            709
```

<210> 2
<211> 2130
<212> DNA
<213> Homo sapience

<400> 2

```
        10         20         30         40         50         60
atgggaccca ggatagggcc agcgggtgag gtaccccagg taccagacaa ggaaaccaaa   60

gccacaatgg gcacagaaaa cacacctgga ggcaaagcca gcccagaccc tcaggacgtg  120

cggccaagtg tgttccataa catcaagctg ttcgttctgt gccacagcct gctgcagctg  180

gcgcagctca tgatctccgg ctacctaaag agctccatct ccacagtgga gaagcgcttc  240

ggcctcttca gccagacgtc ggggctgctg gcctccttca acgaggtggg gaacacagcc  300

ttgattgtgt ttgtgagcta ttttggcagc cgggtgcacc gaccccgaat gattggctat  360

ggggctatcc ttgtggcccc ggcgggcctg ctcatgactc tcccgcactt catctcggag  420

ccataccgct acgacaacac cagccctgag gatatgccac aggacttcaa ggctttccctg  480

tgcctgccca caacctcggc cccagcctcg gccccctcca atggcaactg ctcaagctac  540

acagaaaccc agcatctgag tgtggtgggg atcatgttcg tggcacagac cctgctgggc  600
```

gtgggcgggg tgcccattca gccccttggc atctcctaca tcgatgactt tgcccacaac 660

agcaactcgc ccctctacct cgggatcctg tttgcagtga ccatgatggg gccaggcctg 720

gccttgggc tgggcagcct catgctgcgc ctttatgtgg acattaacca gatgccagaa 780

ggtggtatca gcctgaccat aaaggacccc cgatgggtgg gtgcctggtg gctgggtttc 840

ctcatcgctg ccggtgcagt ggccctggct gccatcccct actttttctt ccccaaggaa 900

atgcccaagg aaaaacgtga gcttcagttt cggcgaaagg tcttagcagt cacagactca 960

cctgccagga agggcaagga ctctccctct aagcagagcc ctggggagtc cacgaagaag 1020

caggatggcc tagtccagat tgcaccaaac ctgactgtga tccagttcat taaagtcttc 1080

cccagggtgc tgctgcagac cctacgccac cccatcttcc tgctggtggt cctgtcccag 1140

gtatgcttgt catccatggc tgcgggcatg gccacctttc tgcccaagtt cctggagcgc 1200

cagttttcca tcacagccct ctacgccaac ctgctcatcg gctgccttc ctttccttcg 1260

gtcatcgtgg gcatcgtggt gggtggcgtc ctggtcaagc ggctccacct gggccctgtg 1320

ggatgcggtg ccctttgcct gctggggatg ctgctgtgcc tcttcttcag cctgccgctc 1380

ttctttatcg gctgctccag ccaccagatt gcgggcatca cacaccagac cagtgcccac 1440

cctgggctgg agctgtctcc aagctgcatg gaggcctgct cctgcccatt ggacggcttt 1500

aaccctgtct gcgaccccag cactcgtgtg gaatacatca cacctgcca cgcaggctgc 1560

tcaagctggg tggtccagga tgctctggac aacagccagg ttttctacac caactgcagc 1620

tgcgtggtgg agggcaaccc cgtgctggca ggatcctgcg actcaacgtg cagccatctg 1680

gtggtgccct tcctgctcct ggtcagcctg ggctcggccc tggcctgtct cacccacaca 1740

ccctccttca tgctcatcct aagaggagtg aagaaagaag acaagacttt ggctgtgggc 1800

atccagttca tgttcctgag gattttggcc tggatgccca gccccgtgat ccacggcagc 1860

gccatcgaca ccacctgtgt gcactgggcc ctgagctgtg ggcgtcgagc tgtctgtcgc 1920

tactacaata atgacctgct ccgaaaccgg ttcatcggcc tccagttctt cttcaaaaca 1980

ggttctgtga tctgcttcgc cttagttttg gctgtcctga ggcagcagga caaagaggca 2040

aggaccaaag agagcagatc cagccctgcc gtagagcagc aattgctagt gtcggggcca 2100

gggaagaagc cagaggatic ccgagigiga 2130

<210> 3
<211> 4083
<212> DNA
<213> Homo sapience

<400> 3

aagigaccca gggagacaaa caciiggaga iaciiggggc igagiiigag caagaciccc 60

iaaccigigi ciggacaagi cigaigicci gigiggccca agaagaacig accccgigic 120

iggagciccc accgiiaiig caicccigci giggcicacc igcigcigic iccaggagcc 180

ccigagaaga iiigccicci ciccccigci aagciccagg iccigagaii gaaiiagggg 240

ciggagcica cigcacicca gcagic 266

aig gga ccc agg aia ggg cca gcg ggi gag gia ccc cag gia cca	311
Met Gly Pro Arg Ile Gly Pro Ala Gly Glu Val Pro Gln Val Pro	
5 10 15	
gac aag gaa acc aaa gcc aca aig ggc aca gaa aac aca cci gga	356
Asp Lys Glu Thr Lys Ala Thr Met Gly Thr Glu Asn Thr Pro Gly	
20 25 30	
ggc aaa gcc agc cca gac cci cag gac gig cgg cca agi gig iic	401
Gly Lys Ala Ser Pro Asp Pro Gln Asp Val Arg Pro Ser Val Phe	
35 40 45	
cai aac aic aag cig iic gii cig igc cac agc cig cig cag cig	446
His Asn Ile Lys Leu Phe Val Leu Cys His Ser Leu Leu Gln Leu	
50 55 60	
gcg cag cic aig aic icc ggc iac cia aag agc icc aic icc aca	491
Ala Gln Leu Met Ile Ser Gly Tyr Leu Lys Ser Ser Ile Ser Thr	
65 70 75	
gig gag aag cgc iic ggc cic icc agc cag acg icg ggg cig cig	536
Val Glu Lys Arg Phe Gly Leu Ser Ser Gln Thr Ser Gly Leu Leu	
80 85 90	
gcc icc iic aac gag gig ggg aac aca gcc iig aii gig iii gig	581
Ala Ser Phe Asn Glu Val Gly Asn Thr Ala Leu Ile Val Phe Val	
95 100 105	
agc iai iii ggc agc cgg gig cac cga ccc cga aig aii ggc iai	626
Ser Tyr Phe Gly Ser Arg Val His Arg Pro Arg Met Ile Gly Tyr	
110 115 120	
ggg gci aic cii gig gcc cig gcg ggc cig cic aig aci cic ccg	671
Gly Ala Ile Leu Val Ala Leu Ala Gly Leu Leu Met Thr Leu Pro	
125 130 135	
cac iic aic icg gag cca iac cgc iac gac aac acc agc cci gag	716
His Phe Ile Ser Glu Pro Tyr Arg Tyr Asp Asn Thr Ser Pro Glu	
140 145 150	

```
gat atg cca cag gac ttc aag gct tcc ctg tgc ctg ccc aca acc        761
Asp Met Pro Gln Asp Phe Lys Ala Ser Leu Cys Leu Pro Thr Thr
                155                 160                 165
tcg gcc cca gcc tcg gcc ccc tcc aat ggc aac tgc tca agc tac        806
Ser Ala Pro Ala Ser Ala Pro Ser Asn Gly Asn Cys Ser Ser Tyr
                170                 175                 180
aca gaa acc cag cat ctg agt gtg gtg ggg atc atg ttc gtg gca        851
Thr Glu Thr Gln His Leu Ser Val Val Gly Ile Met Phe Val Ala
                185                 190                 195
cag acc ctg ctg ggc gtg ggc ggg gtg ccc att cag ccc ttt ggc        896
Gln Thr Leu Leu Gly Val Gly Gly Val Pro Ile Gln Pro Phe Gly
                200                 205                 210
atc tcc tac atc gat gac ttt gcc cac aac agc aac tcg ccc ctc        941
Ile Ser Tyr Ile Asp Asp Phe Ala His Asn Ser Asn Ser Pro Leu
                215                 220                 225
tac ctc ggg atc ctg ttt gca gtg acc atg atg ggg cca ggc ctg        986
Tyr Leu Gly Ile Leu Phe Ala Val Thr Met Met Gly Pro Gly Leu
                230                 235                 240
gcc ttt ggg ctg ggc agc ctc atg ctg cgc ctt tat gtg gac att       1031
Ala Phe Gly Leu Gly Ser Leu Met Leu Arg Leu Tyr Val Asp Ile
                245                 250                 255
aac cag atg cca gaa ggt ggt atc agc ctg acc ata aag gac ccc       1076
Asn Gln Met Pro Glu Gly Gly Ile Ser Leu Thr Ile Lys Asp Pro
                260                 265                 270
cga tgg gtg ggt gcc tgg tgg ctg ggt ttc ctc atc gct gcc ggt       1121
Arg Trp Val Gly Ala Trp Trp Leu Gly Phe Leu Ile Ala Ala Gly
                275                 280                 285
gca gtg gcc ctg gct gcc atc ccc tac ttc ttc ttc ccc aag gaa       1166
Ala Val Ala Leu Ala Ala Ile Pro Tyr Phe Phe Phe Pro Lys Glu
                290                 295                 300
atg ccc aag gaa aaa cgt gag ctt cag ttt cgg cga aag gtc tta       1211
Met Pro Lys Glu Lys Arg Glu Leu Gln Phe Arg Arg Lys Val Leu
                305                 310                 315
gca gtc aca gac tca cct gcc agg aag ggc aag gac tct ccc tct       1256
Ala Val Thr Asp Ser Pro Ala Arg Lys Gly Lys Asp Ser Pro Ser
                320                 325                 330
aag cag agc cct ggg gag tcc acg aag aag cag gat ggc cta gtc       1301
Lys Gln Ser Pro Gly Glu Ser Thr Lys Lys Gln Asp Gly Leu Val
                335                 340                 345
cag att gca cca aac ctg act gtg atc cag ttc att aaa gtc ttc       1346
Gln Ile Ala Pro Asn Leu Thr Val Ile Gln Phe Ile Lys Val Phe
                350                 355                 360
ccc agg gtg ctg ctg cag acc cta cgc cac ccc atc ttc ctg ctg       1391
Pro Arg Val Leu Leu Gln Thr Leu Arg His Pro Ile Phe Leu Leu
                365                 370                 375
gtg gtc ctg tcc cag gta tgc ttg tca tcc atg gct gcg ggc atg       1436
Val Val Leu Ser Gln Val Cys Leu Ser Ser Met Ala Ala Gly Met
                380                 385                 390
gcc acc ttc ctg ccc aag ttc ctg gag cgc cag ttt tcc atc aca       1481
Ala Thr Phe Leu Pro Lys Phe Leu Glu Arg Gln Phe Ser Ile Thr
```

```
                395                 400                 405
gcc tcc tac gcc aac ctg ctc atc ggc tgc ctc tcc ttc cct tcg      1526
Ala Ser Tyr Ala Asn Leu Leu Ile Gly Cys Leu Ser Phe Pro Ser
                410                 415                 420
gtc atc gtg ggc atc gtg gtg ggt ggc gtc ctg gtc aag cgg ctc      1571
Val Ile Val Gly Ile Val Val Gly Gly Val Leu Val Lys Arg Leu
                425                 430                 435
cac ctg ggc cct gtg gga tgc ggt gcc ctt tgc ctg ctg ggg atg      1616
His Leu Gly Pro Val Gly Cys Gly Ala Leu Cys Leu Leu Gly Met
                440                 445                 450
ctg ctg tgc ctc ttc ttc agc ctg ccg ctc ttc ttt atc ggc tgc      1661
Leu Leu Cys Leu Phe Phe Ser Leu Pro Leu Phe Phe Ile Gly Cys
                455                 460                 465
tcc agc cac cag att gcg ggc atc aca cac cag acc agt gcc cac      1706
Ser Ser His Gln Ile Ala Gly Ile Thr His Gln Thr Ser Ala His
                470                 475                 480
cct ggg ctg gag ctg tct cca agc tgc atg gag gcc tgc tcc tgc      1751
Pro Gly Leu Glu Leu Ser Pro Ser Cys Met Glu Ala Cys Ser Cys
                485                 490                 495
cca ttg gac ggc ttt aac cct gtc tgc gac ccc agc act cgt gtg      1796
Pro Leu Asp Gly Phe Asn Pro Val Cys Asp Pro Ser Thr Arg Val
                500                 505                 510
gaa tac atc aca ccc tgc cac gca ggc tgc tca agc tgg gtg gtc      1841
Glu Tyr Ile Thr Pro Cys His Ala Gly Cys Ser Ser Trp Val Val
                515                 520                 525
cag gat gct ctg gac aac agc cag gtt ttc tac acc aac tgc agc      1886
Gln Asp Ala Leu Asp Asn Ser Gln Val Phe Tyr Thr Asn Cys Ser
                530                 535                 540
tgc gtg gtg gag ggc aac ccc gtg ctg gca gga tcc tgc gac tca      1931
Cys Val Val Glu Gly Asn Pro Val Leu Ala Gly Ser Cys Asp Ser
                545                 550                 555
acg tgc agc cat ctg gtg gtg ccc ttc ctg ctc ctg gtc agc ctg      1976
Thr Cys Ser His Leu Val Val Pro Phe Leu Leu Leu Val Ser Leu
                560                 565                 570
ggc tcg gcc ctg gcc tgt ctc acc cac aca ccc tcc ttc atg ctc      2021
Gly Ser Ala Leu Ala Cys Leu Thr His Thr Pro Ser Phe Met Leu
                575                 580                 585
atc cta aga gga gtg aag aaa gaa gac aag act ttg gct gtg ggc      2066
Ile Leu Arg Gly Val Lys Lys Glu Asp Lys Thr Leu Ala Val Gly
                590                 595                 600
atc cag ttc atg ttc ctg agg att ttg gcc tgg atg ccc agc ccc      2111
Ile Gln Phe Met Phe Leu Arg Ile Leu Ala Trp Met Pro Ser Pro
                605                 610                 615
gtg atc cac ggc agc gcc atc gac acc acc tgt gtg cac tgg gcc      2156
Val Ile His Gly Ser Ala Ile Asp Thr Thr Cys Val His Trp Ala
                620                 625                 630
ctg agc tgt ggg cgt cga gct gtc tgt cgc tac tac aat aat gac      2201
Leu Ser Cys Gly Arg Arg Ala Val Cys Arg Tyr Tyr Asn Asn Asp
                635                 640                 645
ctg ctc cga aac cgg ttc atc ggc ctc cag ttc ttc ttc aaa aca      2246
```

```
Leu Leu Arg Asn Arg Phe Ile Gly Leu Gln Phe Phe Phe Lys Thr
                650                 655                 660
ggt tct gtg atc tgc ttc gcc tta gtt ttg gct gtc ctg agg cag      2291
Gly Ser Val Ile Cys Phe Ala Leu Val Leu Ala Val Leu Arg Gln
                665                 670                 675
cag gac aaa gag gca agg acc aaa gag agc aga tcc agc cct gcc      2336
Gln Asp Lys Glu Ala Arg Thr Lys Glu Ser Arg Ser Ser Pro Ala
                680                 685                 690
gta gag cag caa ttg cta gtg tcg ggg cca ggg aag aag cca gag      2381
Val Glu Gln Gln Leu Leu Val Ser Gly Pro Gly Lys Lys Pro Glu
                695                 700                 705
gat tcc cga gtg tga                                             2396
Asp Ser Arg Val
            709
```

gctgtcttgg ggccccacct ggccaagagt agcagccaca gcagtacctc ctctgagtcc 2456

tttgcccaag attgggtgtc aagagccctg tgttccattc tggctcctcc actaaattgc 2516

tgtgtgactt caggcaagac attgatcctc tctcagcctt tgcttgctag tctgaaccaa 2576

agagttgttt gggcattttgc tgtgttggcc atttctggag caagagggtc ttctttcctc 2636

ttccccccagc cagccagctg tcctgggggcc aggctttcct gggtggaaag aagtatacct 2696

ttccctgggg ccctaggata gcaaagtgag ccatagtggg ccaggctgcc ctccatgctg 2756

ggccccagcc caggtctgca ctcgcctgga tcacctttct tgagccttag ccatctcctg 2816

tcaggtagga atgaacttgc cagccttcag gctcgttcag ctatgaccat ctgtgcggtc 2876

agggtacact cagctctcct ccccaactcc agcagccttt aagaagtgtc ccttggcgc 2936

ccccttggagg cagagcactg agctggaccc tgggtagact cccacaggga ggacggagct 2996

ggcctcagga gtgggacacc cagacttggc agggccttca agaggcctgt gtgggggccc 3056

caggaatcct tagctgaagc ggggagactc actctccatc tcaggaaatt ctagccctg 3116

ccctcaggga gccacggttg agggtgaggc ccaacaccctg ccttagggcc ctgggtgggc 3176

aagtctgggc cctggggtag ggagggagac tcaggcccac acttgggtat tttctaattt 3236

cagacaaaca cacactcagc gcgcactcac tgattcctac acattgccaa gatttcacac 3296

atgtgaccag gggccaccaa agtccctgtg accttgtga ctaggatcct aatttctcta 3356

ttttctccctg ggtgcctggg tctgtgtcac ctggggcagt gtggataatg ttagtttctg 3416

tgacactgtt ttttgggggt ggcaccttggt tctccgatgc ctgggctggt gtcaggccca 3476

ggactgtagt gctgggagca gtaaagctca gctctgtgta atgagtgatg ctatggcttg 3536

ctcgtgtctt atgatccaat cctttttctac atcagccctt gttttgtttt atggctagtc 3596

ttatctggcc tggttatttc cttgcgggga ggagagggtt tgctaatctg ctcccagccc 3656

aacctattac caccccacct cgctgggacc tactgctcgg gaggcagcag acagggagcc 3716

accagcagtg gcttcctggc cctgtgctgg gggtgggggg aagctggggg cacatgtggc 3776

cctgccttc tgagcagctc ccagtgccag ggctttgaga ctttcccaca tgataaaaga 3836

aaagggaggt acagaagttc caatttcctt tttatttttgc tggttggtat ctgtaaatgt 3896

ttaataaata tctgagcatg tatctatcaa cgccaagaat ttcaaagtct ccttcaacaa 3956

tatgaggctt ttaggatgtt tatatttcctt catccctctt gtttcccagg ttttgcaggg 4016

aaaaaaagtc tggaattata gatacagctt attattaaat ttgttcttgc ataaaaaaaa 4076

aaaaaaa 4083

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP99/04352

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[6] C07K 14/47,C12N 15/12//C12N 5/10,C12P 21/02,
(C12P 21/02,C12R 1:91)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[6] C07K 14/47,C12N 15/12,12N 5/10,C12P 21/02,

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
SwissProt/PIR/GeneSeq,Genbank/EMBL/DDBJ/GeneSeq,
WPI(DIALOG),BIOSIS(DIALOG)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,5792851,A(Albert Einstin College of Medicine of Yeshiva University,a Division of Yeshiva University) 11 August, 1998 (11.08.98) (Family: none)	1,2
Y	Journal of Clinical Investigation, Vol.98, no.5 (1996) Lu Run, et al., "Cloning, in vitro expression, and tissue distribution of a human prostaglandin transporter cDNA (hPGT)" see p.1142-1149,(1996)	1,2
Y	Biochemical and Biophysical Research Communications, Vol.246, No.3, (May 29,1998), Lu Run, et al., "Molecular cloning of the gene for human prostaglandin transporter hPGT: Gene organization, promoter activity and chromosomal localization", see p.805-812,	1,2

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 November, 1999 (09.11.99)	24 November, 1999 (24.11.99)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/JP99/04352

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Science, vol.268, No.5212, (1995), Kanai Naoaki et al., "Identification and Characterization of a prostaglandin transporter ", see p.866-869,	1-2